

lost interference count, optionally taken in view of any or all of the Lewin, Culver et al., Roizman, De wind et al. and Keeler references.

The interference count requires a virus that is not able to express VP5 as a result of a mutation in the VP5 gene. The Examiner stated that the newly entered claims differ from the count by specifying alteration of at least two nucleotides in the VP5 start codon, and requiring an additional stop codon in the 5 prime end of the VP5 gene. It is to be noted that claim 32 does not require a stop codon. The Examiner has submitted that it is well known in the art of genetics that single base mutations are "leaky" and reversible and cite the textbook by Lewin. The Examiner concluded that one of ordinary skill in the art of genetics and virology would have been aware that a virus with multiple nucleotide changes would be less likely to revert to the undesired wild-type VP5 coding sequence. Alteration of more than one nucleotide to remove a start codon has been known for years, see for example Culver et al. The Examiner concluded that altering more than one base in the start codon would have been obvious to one of ordinary skill as a known form of mutation preventing expression of a protein, with an obvious advantage of reducing the probability of reversion. The Examiner submitted that, in addition, the use of multi-frame stop codons to prevent expression of an unwanted gene is also commonplace, citing examples in Roizman, column 16, lines 16 - 20; De Wind et al., the passages spanning pages 6 and 7; and Keeler, column 3, line 52 to column 4, line 8. The Examiner concluded that since the lost count requires a virus that is not able to produce VP5 as a result of a mutation in the VP5 gene, it would have been obvious to chose forms of mutation that were known to prevent production of a protein, such as changing two nucleotides in the start codon and introducing a 3-frame set of stop codons. The Examiner concluded that the invention as a whole is *prima facie* obvious absent an unexpected result.

The rejection of claims 33 - 39 for obviousness is respectfully traversed. The prior art does not suggest the presently claimed invention, nor is there anything in the prior art to motivate the skilled practitioner to introduce more than one substitution in the VP5 gene.

The Mundt prior art describes a VP5- virus that is the same as the mutant described in example 1 of the present application. All of the references disclose a single mutation that, they teach, could be used to produce an attenuated vaccine strain. The prior art references directed to VP5- mutants teach the ordinary practitioner that the single base pair substitution provides a useful vaccine strain. There is no indication or suggestion that this strain might revert to the wild-type VP5<sup>+</sup>. Support for this conclusion can be found in the experiment described on page 5 of the Mundt poster, where the VP5- mutant is serial passaged three times in cell culture with no reversion. This and the literature cited by the Examiner suggest that additional mutations would not likely be required.

It was only after these publications were in the literature that Applicants discovered that a VP5- mutant with a single substitution could revert at higher passage levels (6 or more). It was only then, in order to develop a stable attenuated mutant strain, that further substitutions were made, yielding a highly stable mutant strain. The experiments provided in the Declaration by Dr. Mundt submitted herewith illustrate that, even after ten to eighteen passages, the mutant with two substitutions was genetically stable.

In order for the skilled practitioner to find it necessary to develop an attenuated mutant according to the invention, there would have to be a suggestion that a multiple substitution or other mutation would be necessary. Otherwise, the course to follow would a single substitution, which is precisely what is taught in the Mundt publications as well as the lost interference count. To render the presently claimed IBDV mutant with at least

two nucleotide substitutions in the start codon of the VP5 gene obvious, there must be a suggestion in the prior art that it is necessary. In fact, the available prior art, as cited by the Examiner, suggests the opposite, that there is no necessity for developing such a mutated strain. Neither the suggestion nor the motivation is there.

It is respectfully submitted that the textbook reference of Lewin cited by the Examiner does not suggest a high probability of reversion to the wild-type VP5 coding sequence. Lewin defines silent mutations, neutral substitutions, forward mutations and back mutations. He also mentions that a true reversion is the exact reversal of the original mutation, which Applicants later discovered occurred with the VP5 substitution described in the Mundt prior art. To quote Lewin, the paragraph in column 2, on page 73, beginning line 18:

"A forward mutation results from any change that inactivates a gene, whereas a back mutation must restore function to a protein damaged by a particular forward mutation. Thus the demands for back mutation are much more specific than those for forward mutation. The rate of back mutation is correspondingly lower than that for forward mutation, typically by a factor of [approximately] 10."

In his final paragraph, Lewin states "[f]orward mutations occur at a rate of [approximately]  $10^{-6}$  per locus per generation; back mutations are rare. Not all mutations, have an effect on the phenotype."

In view of the specific teachings of Lewin, it can only be said that back mutations are possible but are 10 times less likely to happen with point mutations, as described by the prior art for the single mutation VP5- strain, than the forward mutation. This does not suggest to the skilled practitioner that the VP5- taught by the prior art would likely revert. Lewin clearly does not indicate that it is "well known in the art of genetics that single base mutations are 'leaky' and 'reversible'" as suggested by the Examiner. Lewin teaches that this is not likely to happen.

Culver et al. do disclose substituting two nucleotides to abolish the expression of the coat protein. There is no reason given, however, why two substitutions are introduced into the start codon and no statement is made that this double mutation would result in a more stable mutant than a single substitution. Moreover, Culver et al. address a different problem from the present invention. They were investigating whether the hypersensitive region induced by tobacco mosaic virus is caused by the coat protein or by altered viral RNA (page 755, Abstract and second column, first full paragraph). For this investigation a mutant in which the expression of the coat protein was abolished was prepared. Stability was not an issue considered by Culver et al.

In addition (also in the first full paragraph in column 2 on page 755), it is taught that the hypersensitive reaction inducing mutant TMV25 differs from the parent TMV204 by a single point mutation in the coat protein gene that leads to a single amino acid substitution. This single point mutation was reportedly demonstrated to be responsible for the induction of hypersensitive reaction in the tobacco mosaic virus. No mention is made whether or not the single point mutation was reversible to the parent TMV204. This reference suggests nothing to the ordinary skilled practitioner that would lead one to introduce more than a single point mutation for any purpose, other than the fact that it happened to be done in one embodiment disclosed by Culver et al. But there is no suggestion or teaching of introducing more than a single point mutation for the purpose of achieving stability, and in fact nothing regarding stability or an expectation that stability would be a problem in a single mutation is suggested by Culver et al.

Roizman teaches the modification of a herpes simplex virus by preventing expression of a particular gene encoding an active gene product. The Examiner cited column 16, lines 16 - 20, which reads as follows:

"To eliminate the possibility that a phenotype of R3616 reflects deletion in cryptic open reading frames, a virus was constructed to contain translational stop codons in all three reading frames in the beginning of ICP34.5 coding sequence."

Roizman, therefore, teaches the introduction of translational stop codons in all three reading frames in the beginning of the ICP34.5 coding sequence. It does not suggest that this is necessary for maintaining stability against reversion of the modified strain to the wild-type. Nothing, in fact, is suggested with regard to stability or lack thereof in recombinant herpes simplex viruses. There is nothing to suggest to the ordinary skilled practitioner that an IBDV mutant with a single mutation in the VP5 gene could likely revert to the wild-type while a substitution of at least two nucleotides would prevent it, or that stability is a problem with this type of virus.

Similarly, Keeler, Jr., column 3, line 52 to column 4, line 8, cited by the Examiner teaches an oligonucleotide comprising three translational stop codons in each of the possible three reading frames. Again, no suggestion of the problem of instability and reversion is found, and no suggestion of introducing multiple substitutions in order to prevent reversion is suggested. Although reversion is mentioned in a general sense under Background of the Invention, columns 1 and 2, there is no discussion of a reversion problem with deletion mutants in the invention of Keeler, Jr., or that this problem was solved by multiple substitutions. It may be noted that Keeler, Jr. mentions "IOTV mutants according to the invention can also be obtained by inserting a nucleic acid sequence into the TK coating region thereby preventing the expression of a functional TK enzyme. Such a nucleic acid sequence can inter alia be an oligonucleotide, for example of about 10-60 bp, preferably also containing one or more translational stop codons, or a gene encoding a polypeptide." Column 3, lines 52 - 59, (emphasis added). No distinction is given for electing one stop codon or

more than one stop codon, at least not for the purpose of preventing reversion or stability in general.

In view of the above it is clear from the prior art that reversion to the wild-type occurs, but it is 10 times less likely to occur than forward mutation. This is taught by Lewin. Examples of multiple mutation and single point mutations with reference to modified virus strain are taught by Culver et al. without an indication that the single point mutations are less stable. The other references teach that both single and multiple mutations are possible. However, in none of the references, is there the suggestion of a general problem of lack of stability and reversion to wild-type, only that it is possible (Lewin). None of the other references cited by the Examiner even mention this as an issue.

The last count is directed to an IBDV mutant that is not able to produce a VP5 protein as a result of a mutation in the VP5 gene. The Mundt publications teach a VP5- strain comprising a single substitution. None of these suggest that there are problems with stability in the Mundt mutant strain of the prior art. This problem was discovered by Applicants and was solved by preparing a modified strain comprising a substitution of at least two nucleotides of the start codon of the VP5 gene.

In the absence of the suggestion that this problem existed or was even likely, particularly with the IBDV VP5- strain, even though the prior art disclosed a VP5- strain having a single substitution, there would have been no motivation to make the present invention. Accordingly, it is believed that claims 32 - 39 are in condition for allowance. Favorable action is solicited.

Submitted herewith is a Declaration by Dr. Egbert Mundt, one of the inventors for the present application, providing comparative results with respect to serially passaging the recombinant IBDV constructs disclosed in example 1 of the present application. The VP5<sup>-</sup> construct, with a single substitution, and

the VP5<sup>-</sup> -2 construct, with two substitutions, are compared with respect to stability on serial passaging. The single substitution mutant reverts to express a positive VP5 protein beginning as early as the sixth passage, while the modified strain having two substitutions demonstrated stability through the eighteenth passage.

Should the Examiner consider that a conference would be helpful in advancing the prosecution of this application, she is invited to telephone the Applicants' attorney at the number below.

Respectfully submitted,

  
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DOLUTTICKEN-AMENDMENT